

The Origin and Development of the
Embryo Sac and Embryo of *Dendrophthora opuntioides* (L.) Eich.
and *D. gracile* Eich.

DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS
UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

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1911

ACKNOWLEDGMENT

TO PROFESSOR DUNCAN S. JOHNSON THE WRITER WISHES TO EXPRESS
HIS SINCERE THANKS AND APPRECIATION FOR
HELPFUL CRITICISM AND ADVICE



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REPRINTED FROM

THE

BOTANICAL GAZETTE

AUGUST 1913

THE ORIGIN AND DEVELOPMENT OF THE EMBRYO
SAC AND EMBRYO OF DENDROPHTHORA
OPUNTIOIDES AND D. GRACILE. I¹

HARLAN HARVEY YORK

(WITH SIX FIGURES AND PLATES V AND VI)

Introduction

The Loranthaceae are parasitic plants that occupy a place of special interest among the dicotyledons. Since they are very peculiar, not only in their mode of life, but also in the structure of their reproductive organs, they have attracted much attention from plant morphologists. Although a considerable number of studies of the group have been made, their morphology and physiology are quite inadequately known. Our knowledge even of the European species of *Loranthus*, *Arceuthobium*, and *Viscum*, those that have been studied most, is as yet incomplete.

The Loranthaceae comprise more than 600 species and are confined largely to the tropics. Aside from the European species of the genera just mentioned, the development of these plants has been most fully studied in certain species of the same genera occurring in Java. In spite of the wide distribution of the genus *Phoradendron* in the southeastern United States and the occurrence of scores of other species in tropical America, comparatively little is known of the Loranthaceae of the Western Hemisphere.

¹ Contribution from the Botanical Laboratory of the Johns Hopkins University, no. 26.

The observations of the earlier writers on the reproductive organs of the Loranthaceae were concerned chiefly with the mature flowers and the development of the fruit. Their interpretation of the different floral organs and the relation of these to each other show that they had a very meager understanding of these structures. Nevertheless, some of these observations show considerable accuracy and are therefore worthy of mention.

WILLIAM GRIFFITH (10), in a paper read before the Linnaean Society of London in 1836, gave a brief description of the anatomy of the flowers and the development of the embryo in *Loranthus scurrula*. He asserted that the ovary is "intimately adherent" with the calyx and that some time after the "fall of the corolla a small cellular body appears attached at the base of the ovarian cavity." This structure, which is an elongation of the floral axis, he interpreted as the rudiment of an ovule, at the center of which, in later stages of development of the fruit, an embryo appeared. In a second paper read before this same society in 1843, GRIFFITH (11) described the mature embryo sacs of *Loranthus bicolor* and *Loranthus globosus*, stating that he believed they existed even before pollination. He found a "nipple-shaped process" at the base of the ovarian cavity and thought it might be a continuation of the floral axis. Not being sure of the homology of this process he limited his descriptions to the ovules, which he called embryo sacs, their relations to the pollen tubes, and the subsequent changes in them. His main conclusions are that the ovules in this genus are reduced to embryo sacs, and that "the embryo is a growth from the ends of the continuations of the pollen tubes, outside the anterior ends of the embryo sacs."

SCHLEIDEN (31) regarded the flowers of the Loranthaceae, which he studied in *Viscum album* and *Loranthus* sp., as the "simplest that can exist." He asserts that the two pairs of bracts of the perianth which bear the stamens are "metamorphosed into anthers" and the segments of the perianth in the carpellate flower "have the nature of a calyx." According to him the floral axis is prolonged between the sepals, forming a nucellus or "ovulum nudum." Furthermore, the tip of the nucellus constitutes a stigma, on which the pollen grains are deposited and into the tissue of which

the pollen tubes, containing the "germ of the embryo," grow downward to the embryo sacs, in which the embryos are nourished. He believed that the embryo sacs are formed in the "pith of the peduncle" or the center of the nucellus, and that the berry is to be regarded as a "metamorphosed peduncle." In conclusion, SCHLEIDEN states that "the Loranthaceae show, in a parasitic form, the intervening step between the Coniferae and more highly developed families" of the angiosperms. MEYEN (21) held essentially the same views as SCHLEIDEN.

TREVIRANUS (36) disagreed with SCHLEIDEN and MEYEN, believing that the central portion of the flower of *Viscum album* constitutes an ovary, within the solid tissues of which the embryo sacs arise.

The investigations of HOFMEISTER (13) greatly advanced our knowledge of the floral organs of the Loranthaceae, he being the first to study the origin and development of the flowers. His extremely accurate observations were made on *Loranthus europaeus* and *Viscum album*. In these forms the flowers are axillary in origin and position. According to HOFMEISTER, soon after the sepals and carpels have appeared in *Loranthus* the apex of the floral axis elongates, growing up between the carpels and forming a small cone-shaped mass of tissue which later becomes united with them. He found that the embryo sacs are formed in the elongated floral axis which he regarded as "a naked ovule." Below this "ovule" is a little plate of collenchymatous tissue which he believed to be chalazal in nature. He also found that the floral parts in *Viscum album* originate quite similarly to those in *Loranthus europaeus* except that there is no swelling of the floral axis between the carpels, which finally fuse to form a single mass of tissue. The embryo sacs, which are usually two in number, arise from a group of cells in the tip of the floral axis.

VAN TIEGHEM (37), from his observations on *Viscum album*, was led to believe that the central mass of tissue of the flower is apical in origin and homogeneous throughout its extent. He described it as being formed by two carpellary leaves, each having its own vascular supply from the peduncle, and becoming "connate" on their "ventral surfaces." He believed that embryo sacs arise

in the lower half of the carpellary tissue, one or two sacs for each carpel. He interpreted the central parenchymatous tissue as a solid ovary, no special segments being differentiated for the production of ovules.

We are indebted chiefly to TREUB (33) for an accurate account of the development and a clear interpretation of the reproductive organs of the Loranthaceae. He found in *Loranthus sphaerocarpus* that the tip of the floral axis grows up between the carpels, forming a mammilliform body, the "mamelon," which becomes united to them along their inwardly projecting margins. Between these lines of union with the carpels the "mamelon" is lobed. There are as many lobes as carpels, which are 3-5 in number. An embryo sac is formed in each lobe of the "mamelon." TREUB interpreted these outgrowths of the "mamelon" as rudimentary ovules, and the central region of the "mamelon" he regarded as a placenta. He found in *Loranthus pentandrus* but a slight elevation of the floral axis between the carpels which he called a "rudimentary placenta" and in which the embryo sacs are formed. TREUB (35) described a still greater reduction in the floral parts of *Viscum articulatum*, in which there is no projecting placenta or "mamelon." The embryo sacs in this species arise from sub-epidermal cells of the sunken apex of the receptacle. JOST (15) described later a similar condition in *Viscum album*.

According to JOHNSON'S (14) account of his observations on *Arceuthobium Oxycedri*, there is at the time of pollination a projection of the floral axis which fills the entire ovarian cavity but does not fuse with its walls. He has shown that in opposite sides of this body two embryo sacs are formed, each of which arises from a hypodermal cell. JOHNSON assigns the same morphological value to the elongated axis that TREUB did for *Loranthus sphaerocarpus*. He describes the anthers as sessile on the segments of the perianth and without vascular bundles.

A few years later, PEIRCE (24), in his studies on *Arceuthobium occidentale*, confirmed JOHNSON'S account of the morphology of the fruit. His observations together with those of YORK (43) on *Phoradendron flavescens* are the most detailed which have been made on the fruit of the American Loranthaceae.

During May and June of 1910, observations were made on 18 different species belonging to 8 different genera of Loranthaceae occurring in Jamaica, and material was collected for a detailed study of their development. From a part of this material the following study was made. This paper, which embodies the results of the beginning of a comparative study of the morphology and physiology of the North American Loranthaceae, embraces an account of the origin and development of the embryo sac and embryo of *Dendrophthora opuntiioides* and *D. gracile*, together with a discussion of the physiological relation existing between the gametophyte and sporophyte of these plants. Although this study may lead to the extension of our knowledge of the life histories and relationships of certain genera of the Loranthaceae, and perhaps to a clearer understanding of the systematic position of this family, one of the most interesting as well as most difficult problems for solution will be the determination of the factors which have caused the striking modification and adaptations in the reproductive organs. The material on which this study was made was killed in medium chromacetic acid and cut in sections 10μ in thickness.

To Professor DUNCAN S. JOHNSON the writer wishes to express his sincere thanks and appreciation for helpful criticism and advice.

Dendrophthora opuntiioides

Dendrophthora opuntiioides (L.) Eich. receives its name from its *Opuntia*-like appearance (text fig. 1). It is a bright yellowish green, glabrous shrub, seldom more than 4 dm. in height. The stems are jointed, constricted at the nodes, and all the joints of a branch are strongly flattened in one plane. The secondary branches arise in this plane in the axils of the reduced scalelike leaves at the nodes. The internodes are usually paddle-shaped, tapering toward the base. The flowers are opposite, isolaterally arranged in spikes which arise from the nodes of the younger portions of the stem in the same manner as the vegetative branches. The spikes thus formed are strongly flattened structures, whose plane of flattening is parallel with that of the stems. The position of a branch as it develops may vary somewhat from the plane in which the stems are flattened, so that all parts of the whole plant do not always lie

in one plane. However, the whole plant is essentially a single isolateral system.

D. opuntioides as observed in Jamaica occurs in well lighted positions at elevations of 2000–5500 ft. above sea-level. It was found most frequently on the following named plants: *Oreopanax*



FIG. 1.—*Dendrophthora opuntioides*: h, branch of host

capitatum Decne. and Planch., *Rhytidophyllum tomentosum* Mart., *Baccharis scoparia* Sw., *Heterotrichum patens* DC., and *Byrsonima* sp.

Dendrophthora gracile

Dendrophthora gracile Eich. is dark yellowish green in color and is quite similar in appearance and mode of branching to *D. opuntioides*. The branches, however, are often almost terete, and the tendency toward an isolateral arrangement of the branches is less prominent than in *D. opuntioides*. The flowers may be

arranged as in the latter, but they often show a tendency toward a decussate or whorled arrangement. The plants are distinctly dioecious, both forms being exactly alike in external appearance, except that the staminate flowers are usually more densely crowded on the spikes. The anthers are unilocular and sessile on the segments of the perianth. *D. gracile* was found at altitudes of 5000–6500 ft., in well exposed places. It was observed mostly on *Vaccinium meridionale*.

The spikes

Since these two species of *Dendrophthora* are so much alike, the following descriptions, which are drawn primarily from *D. opuntioides*, will be understood to apply to both unless otherwise stated. Each spike is usually composed of two or three internodes, and at the base of each internode there is a pair of connate bracts (figs. 3, 6). Primary growth of this axis is accomplished by the activity of an apical group of initials (fig. 2). There is considerable secondary elongation of the spike, due to an intercalary growth zone at the base of each internode.

A single pair of flowers is usually borne in each internode with the exception of the basal one (fig. 3). They arise laterally just below the apex of the spike, and are at no time closely associated with the bracts at the base of the internode, that is, they do not seem to be axillary in position (figs. 4, 5). In other spikes, which occur less frequently, there are often two or three pairs of flowers on each internode; of these the terminal pair is evidently older, and if three pairs are present, the basal pair is youngest of all (fig. 6). The appearance of these internodes, when the flowers are nearly mature, suggests that the second and third pairs have arisen successively during the intercalary growth of the internode, and that they have arisen from the young tissue at the base of the internode while this portion was still surrounded by the subtending bracts. This phenomenon, which seems unusual in these species, was noted by EICHLER (7) in *Dendrophthora Mancinellae* Eichl., and is a characteristic feature, for example, of the inflorescence of *Phoradendron crassifolium* Pohl., where the number of flowers on each internode is much greater than in *D. opuntioides*.

The vascular system of the spike is comparatively simple. From each spike there enter the stem 6-14 vascular bundles, which are arranged as in a typical dicotyledonous stem except that there is no interfascicular cambium (text fig. 7). Growth in thickness is entirely by the divisions of the fascicular cambium and the parenchyma cells between the bundles. A single leaf trace passes from each segment of the perianth into the axis of inflorescence. The paths of the bundles were traced in detail through the basal internode, the first node above the stem, and the first pair of flowers. One bundle, which is entirely free from the others, extends from each of the two connate bracts into the axis. The first pair of bundles on the right and left sides of the axis

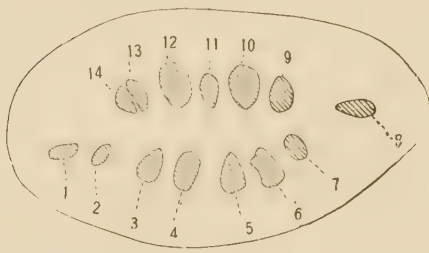


FIG. 7

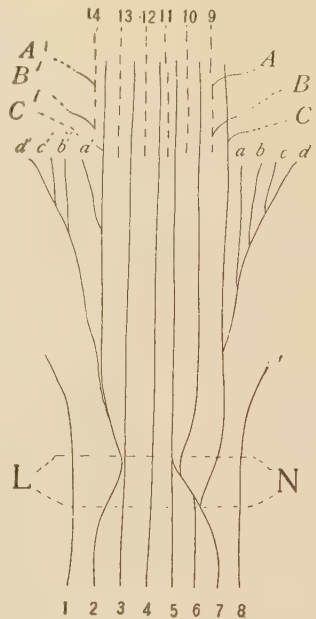


FIG. 8

FIGS. 7, 8.—Fig. 7, cross-section near base of spike, indicating arrangement of vascular bundles; fig. 8, diagram showing arrangement of vascular bundles in lateral view in the first node and first pair of flowers of fig. 3: 1, 2, 3, 4, 5, 6, 7, 8, same as in fig. 7; LN, level of first node; 1, 8, bundles entering the bracts; a, b, c, d, a', b', c', d', tracheid strands entering the swollen portion of the inflorescence axis (fig. 4, c) about flower; A, B, C, A', B', C', bundles entering inflorescence axis from the flower (figs. 13, v, 20, v); 9, 10, 11, 12, 13, 14, as in fig. 7.

furnishes the traces for the swollen collar of the stem surrounding the flower. With these same bundles the vessels of the flowers are connected (text figs. 7, 8). The bundles beyond the first pair of flowers were not followed closely, but their distribution is apparently similar to the portion of the system here figured and described.

The flower

The earliest stage obtained in the development of the flower shows that it is not axillary in origin, but is initiated by a bulging out of the periblem of the inflorescence axis some distance above the axil of the subtending bract (figs. 4, 5). The flowers of *Rhopalocnemis phalloides* have a similar origin, as was shown by LOTSY (20). Following such a stage, the surrounding tissues of the incipient floral axis expand rapidly, which results in it becoming almost completely buried within the axis of the young spike (figs. 4, 9). Two cycles of floral segments develop acropetally on the young axis of the flower. The outer consists of three parts, which are the segments of the perianth; while the inner is composed of two seg-

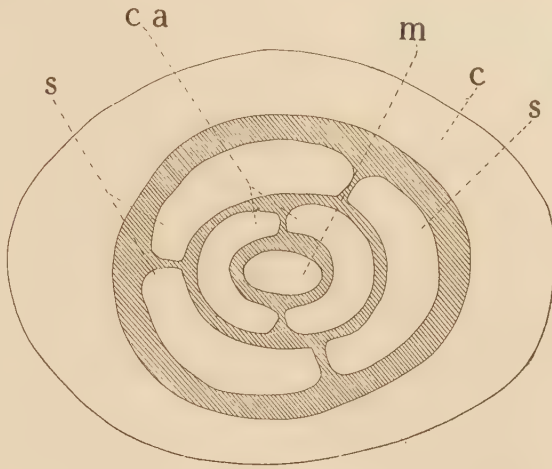


FIG. 12a.—Diagrammatic cross-section of flower taken at AB, fig. 12: *m*, floral apex or "mamelon"; *ca*, carpels; *s*, sepals; *c*, swollen part of inflorescence axis about flower.

ments, the carpels. The perianth completely covers the apex of the floral axis before the carpels have begun to develop (fig. 10). The two carpels arise as distinct primordia, and upon elongating inclose the moundlike apex of the floral axis between them (fig. 11). At this period of development the flower is still sunken in the axis of the spike, and its various parts are clearly distinct from one another (fig. 12, text fig. 12a). The apical growth of the floral axis is never active, and the outer region, composed of torus and the basal portion of the carpels, now grows rapidly, and thus the axis with the narrow ovarian cavity about it becomes deeply sunken within the torus (fig. 13). According to GOEBEL (9), there is a somewhat similar uprising of the torus and carpels

in the early development of the flower of *Pyrus Malus*. The lateral walls of the ovarian cavity are thus lined on the inside by carpellary tissue and the ovary is distinctly epigynous. Meanwhile the moundlike apex of the floral axis has been slowly developing and has come to occupy the entire ovarian cavity. It is in close contact with the walls of the cavity but never becomes united with them. In form, it is a compressed knob, flattened in the same plane with the spike. A transverse mid-section of the knob is oval in outline (fig. 14). A longitudinal section perpendicular to its broad surface is finger-shaped, while a longitudinal section in the plane of flattening shows the knob form (figs. 15, 17, 18).

HOFMEISTER in *Loranthus europaeus* and TREUB in *L. sphaerocarpus* found that the floral axis elongates between the carpels after they have appeared. According to BAILLON (1), the apical part of the floral axis is present throughout the development of the carpellate flower in *Arceuthobium Oxycedri*, just as we have seen it to be in *Dendrophthora*.

The megasporangium (nucellus?)

The floral apex is composed entirely of parenchymatous tissue and has a distinct epidermis. Parallel with the enlargement of the floral axis the cells subjacent to the sporogenous cells divide by periclinal and anticlinal walls (fig. 16). Later, when the uninucleate sac has been formed, it is partially surrounded by a tissue of a few cells in thickness, which has resulted from the divisions of these underlying cells. The contents of the cells of this tissue contain no starch, but have dense cytoplasm, their walls are thicker, and they stain more darkly than the cells of the other portions of the axis (fig. 17). The tissue thus formed about the young gametophyte is apparently nutritive in function, and may be regarded as the equivalent of a nucellus. TREUB has shown that a similar tissue is formed about the archesporial cells in *Loranthus sphaerocarpus*, but the cells are in this case filled with starch. In *Arceuthobium Oxycedri* there is apparently no nucellus formed as in the above mentioned cases. By the time the two-nucleate sacs are developed, the cells of the nucellus have become much enlarged and the axis has become distinctly lobed (figs.

17, 18). A similar though somewhat more marked lobing occurs in *Loranthus sphaerocarpus* (TREUB 33). The epidermal cells immediately above the sporogenous cells divide by one or two periclinal walls, forming a small cap of cells which is probably to be regarded as the remnant of an integument (fig. 18). WARMING (38), in studying the development of the reproductive organs of *Thesium*, found that the contents of the epidermal cells above the apex of the nucellus were more densely granular than the remaining epidermal cells, and that the epidermal cells surrounding these granular ones divide by periclinal walls, forming a tissue about three cells in thickness, which he regarded as vestiges of integuments.

The question of the interpretation of the elongated floral axis, the "mamelon," has been a puzzling one. According to HOFMEISTER, who first worked out its development in *Loranthus europaeus*, it is a "naked ovule" in which there are several groups of archesporial cells present. BAILLON also gave the same interpretation to this body in *Arceuthobium*. TREUB believed that the "mamelon" is a growth of the floral axis in which the separate nucelli represent rudiments of ovules. In reference to HOFMEISTER'S idea, TREUB asserts that there is no reason to consider this hemispherical process as an ovule reduced to its nucellus. Nowhere are groups of embryo sac mother cells formed in the lateral part of a nucellus as would be the case in *Loranthus* if the "mamelon" be regarded as an ovule.

Aucune raison ne nous engage à considérer le processus hémisphérique comme un ovule réduit à son nucelle. Nulle part plusieurs groupes de cellules mères de sacs embryonnaires ne naissent dans les parties latérales d'un nucelle, comme cela serait le cas chez le *Loranthus* si le mammelon en litige méritait le rang d'ovule.

Comparing the enlarged floral apex or "mamelon" as seen in the Loranthaceae with that in certain genera of the Santalaceae, we find a striking resemblance and further evidence for the correctness of TREUB'S views. In the early development of the flower of *Thesium divaricatum*, there is a central elongation of the floral axis as in *Dendrophthora opuntiioides* and *D. gracile*. This "mamelon" elongates with the formation of the ovarian cavity and forms a

lobe opposite each of the three carpels. As development continues, these lateral outgrowths enlarge and grow downward along the sides of the axis. These lobes are the ovules. In direction of growth they are anatropous and do not become fused with the placenta along the side of which they grow. Only mere rudiments of integuments are formed, as has been already mentioned. The ovules are thus practically naked. The archesporial cells are sub-epidermal in origin. In *Santalum album* there is a conelike "mamelon" quite similar to that in *Thesium*, but less extensively lobed. The ovules are without integuments. In *Osyris alba* naked ovules are found on a central placenta as in the two preceding examples, except the ovular lobes at first grow downward and then curve upward toward the apex of the placenta.

From this comparison it is evident that TREUB's interpretation of the "mamelon" is correct. The two lateral lobes of the elongated floral axis in *Dendrophthora opuntioides* and *D. gracile* are thus rudimentary ovules borne on a central placenta. The micropylar end of the nucellus is toward the base of the placenta, and the chalazal portion lies in the apical part of the floral axis (fig. 17).

The vascular system of the flower

One vascular trace passes from each of the three segments of the perianth into the inflorescence axis. A fourth vessel may enter the axis from near the base of the placenta, but there are no traces in the placenta. The bundles within the segments of the perianth as a rule never branch. About the time of the origin of the one-celled embryo sac, the cells just below the insertion of the placenta begin to enlarge and the walls become unequally thickened. Later there is formed a mass of short tracheids, which are somewhat similar in appearance to water-storage tracheids (fig. 19). This plate of tracheids is analogous to the plate of collenchymatous tissue in *Loranthus europaeus* which HOFMEISTER interpreted as being chalazal in nature. TREUB has shown that a collenchymatous body similar to that described by HOFMEISTER is present in *Loranthus sphaerocarpus*. During the formation of these short tracheids, a series of longer tracheids appear in the outer wall of the ovary at the level of the base of the placenta (text fig. 20, *t*). They

originate thus in or near the zone of elongation of the torus and extend upward from their place of origin, finally fusing with the bundles in the segments of the perianth. Other strands of tracheids pass upward from the plate of tracheids (text fig. 20, *tp*) near the

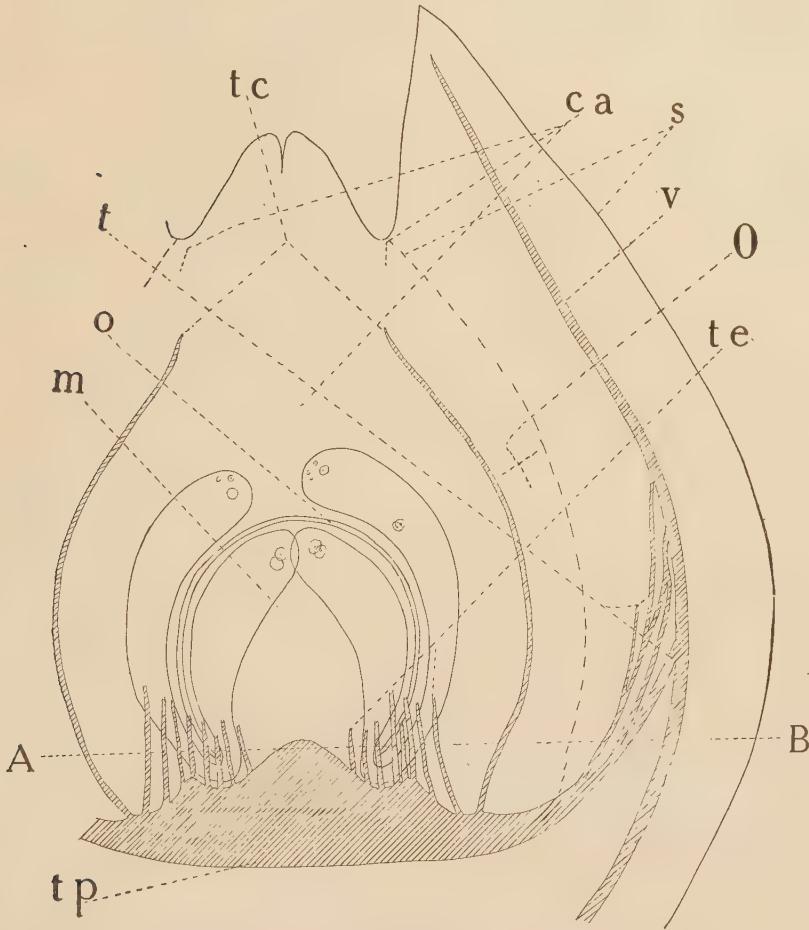


FIG. 20.—Diagrammatic longitudinal section of flower at maturity of embryo sac; *te*, strands of tracheids in inner walls of carpels; *t*, strands of tracheids connecting vascular bundles of the sepals with tracheid tissue beneath "mamelon"; *v*, vascular bundle of sepal; *tp*, plate of water tracheid tissue beneath "mamelon"; *tc*, strands of tracheids in middle region of carpels; *s*, tissue of sepal; *ca*, tissue of carpels; *m*, "mamelon"; *o*, cavity of ovary.

inner walls of the ovary, and by the time of the formation of the mature sac some of them reach almost halfway to the level of the apex of the placenta (text figs. 20, 21, *te*). By the completion of the development of the embryo sac, there are 10–12 vascular traces distributed between the three main bundles of the perianth, with which they have become united (text fig. 21, *t*). Besides uniting with

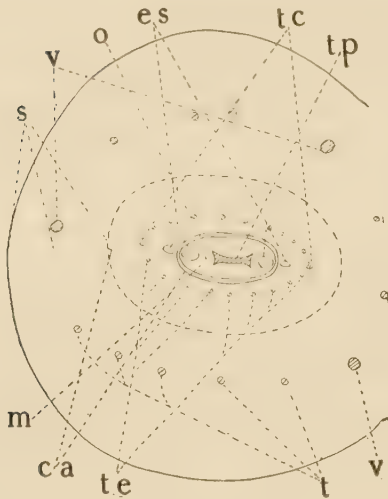


FIG. 21.—Diagrammatic cross-section of flower taken along line *AB* in fig. 20, showing vascular system: *v*, same as *v* in fig. 20 and *A*, *B*, *C*, and *A'*, *B'*, *C'* in fig. 8; *t*, *tp*, *tc*, *s*, *ca*, *m*, *o*, as in fig. 20; *es*, embryo sacs.

the bundles of the perianth, their branches anastomose with each other, so that in the mature fruit there is an intricate network of vessels within the fleshy pericarp. The vessels, formed in the inner wall of the carpels, constitute their vascular system. The embryo sacs are in close proximity to some of these vessels, while later the endosperm comes to be in direct contact with them. In the ripe fruit the outer coat of the lower half of the seed is formed of remnants of these tracheids.

Two small strands of tracheids pass upward from the vascular complex in the lower part of the carpels to the base

of the style. These strands lie in the plane of flattening of the floral axis (text figs. 20, 21, *tc*). The tissue between these strands is eventually displaced by the endosperm, which then lies in contact with them.

Development of the megaspore

Previous to the upward growth of the torus, as explained above, the archesporial cells become organized. There are two in each "mamelon," hypodermal in origin and located at the poles of the mid-horizontal diameter of the enlarged floral axis (fig. 23). They

are first distinguished by their larger nuclei and more densely staining protoplasm, and without cutting off tapetal cells they develop directly into what may be called megaspore mother cells (fig. 16).

JOHNSON'S account of *Arceuthobium Oxycedri* states that the primary archesporial cell divides into two cells. The upper one becomes the primary tapetal cell, which later divides by an anticlinal wall into two cells, while the lower cell becomes "the mother cell of the embryo sac." TREUB'S work shows that no tapetal cells are formed in *Loranthus sphaerocarpus*. He found that the archesporial cell in *Viscum articulatum* does divide into two cells, the lower one developing directly into an embryo sac. It is not possible to determine the character of the upper cell, since it is not known where reduction division occurs.

Preceding division the megaspore mother cell becomes very much enlarged (fig. 16). Later the chromatin thread is organized, becomes thickened, and forms a loose mass, the synaptic knot. Following synapsis, it segments into chromosomes, which later become arranged on the spindle. From two counts of the chromosomes in the dividing megaspore mother cell nuclei of two different ovules at this stage of development, it is apparent that 18-20 chromosomes pass to each pole of the spindle. The same number of chromosomes was found in dividing cells of the young embryo. Thus it seems that no reduction division takes place; hence this division is a normal vegetative division. Since the nucleus passes through what is apparently a synapsis before dividing, it may be regarded as analogous to a megaspore nucleus of the usual type. The two cells resulting from the division of the so-called megaspore mother cell in *Dendrophthora* are separated by a thin wall and lie just beneath the epidermis of the nucellus (fig. 24). The one toward the micropylar region of the nucellus degenerates, while the one in the chalazal portion gives rise to the embryo sac (fig. 25).

In the seed plants in which parthenogenesis is said to occur there is a tendency toward a reduction of the number of divisions of the so-called megaspore mother cell. Four megaspores are formed in *Thalictrum* and *Eualchemilla*. Only two occur in *Taraxacum*

and *Hieracium*, while in *Antennaria* and *Wikstroemia* they have been omitted entirely. The archesporial cell develops directly into an embryo sac in *Balanophora elongata* and *Elastonema acuminatum* investigated by TREUB, and in *Balanophora globosa* studied by LOTSY. The authors claim that the embryo develops apogamously.

In *Arceuthobium Oxycedri*, according to JOHNSON, two small cells are cut off from the lower end of the "embryo sac mother cell." Of the three cells thus formed, the uppermost one, that is, the one toward the micropyle, becomes the one-celled stage of the embryo sac. TREUB has described the same for *Loranthus sphaerocarpus*. He refers to the two lower cells as "anticlines." These authors make no reference to megaspore formation, nor is the place of the reduction division known, yet we may assume that the cells just mentioned are megaspores.

The development of the embryo sac

Two embryo sacs are formed in each ovary, one from each chalazal nucleus, resulting from the division of the megaspore mother cell. Since the development of the two sacs is practically the same, we need follow the history of but one. The cell which gives rise to the gametophyte may be said to become the one-celled sac, and on dividing the two-nucleate sac is formed (figs. 14, 17, 26). No traces of cell walls were observed between these two nuclei. Their division results in the formation of the four-nucleate sac, in which the two nuclei at each pole of the sac are sister nuclei (fig. 27). Simultaneously with the formation of the one-nucleate sac, starch and other food materials are being laid down within its cavity, and by the completion of the four-nucleate stage it is often so densely filled with these substances that the nuclei are almost completely hidden (fig. 28). This is especially true of the sacs of *Dendrophthora gracile*. Ovules of this plant were found in which the nuclei of the sac were either degenerating or had entirely disappeared as a result of the abundant storage of food. In some examples one of the nucelli had been replaced by a cavity filled with food materials (fig. 29). The starch and other organic substances associated with it evidently serve as nourishment for the

further development of the gametophyte, and by the completion of the sac they have usually been entirely consumed. The sac toward the base of the inflorescence as a rule contains a greater amount of stored food substances than its mate, and is the one in which degeneration of the nuclei occurs. No examples were observed where the nuclei in both sacs had begun to disintegrate.

Following this stage, the end of the sac grows almost straight downward in the floral axis until it extends below the level of the insertion of the placenta. It then curves outward into the tissue of the carpel, bends, and grows upward beneath the epidermis of the inner wall of the carpel until the micropylar end of the sac lies almost over the apex of the floral axis (figs. 29, 30). At first it forms a very narrow tube, having a diameter but slightly greater than the width of the cells adjacent to it. It forces its passage through the tissues of the floral axis and the carpel by digesting the cells in front of it. The behavior of the sac suggests strongly that of a pollen tube. GRIFFITH (11), in his observations on the Santalaceae, figured and described the branching of the chalazal portion of the embryo sac of *Santalum* and mentioned that it behaved much like a pollen tube. LLOYD (18) calls attention to the pollen-tubelike behavior of the embryo sac in his studies of the Rubiaceae. The disintegration of the cells indicates clearly the secretion of an enzyme by the tubelike sac. A portion of the protoplasm in this sac seems to be specialized for this purpose, as will be shown later. As the sac advances, it evidently derives its nutriment from the cells immediately surrounding it. In a sense it is a parasite within a parasite, feeding on the tissue through which it moves. The path of growth of the sac brings it into a most advantageous position for obtaining food. It also provides a line of transit through which food is readily transferred to the developing endosperm and embryo. When the sac grows downward, it comes into direct contact with the vessels at the base of the placenta (text fig. 20). As it turns out into the carpels and grows upward, it is in close proximity to vascular traces from which it draws its nourishment. The cells of the region of the carpel through which the sac moves are richer in protoplasm than the surrounding tissue (fig. 31). No starch was found in these cells, yet their general

appearance and reaction to various stains indicate that they are supplied with some substance which serves as food for the embryo sac. Hence the direction of growth of the embryo sac may be regarded in part as a response to chemotactic stimuli. In *Loranthus sphaerocarpus*, according to TREUB, the embryo sac is surrounded by a sheath consisting of a single layer of cells which are filled with starch. During the development of the long arm from the base of the sac, the portion within the floral axis has been slowly enlarging and advancing upward. The sac as now seen is shaped like a hook, the short arm of which is within the floral axis, while the long club-shaped portion lies wholly within the tissue of the carpel.

The history of the formation of the nuclei of the sac and their arrangement within it are no less interesting to follow than the form of the sac itself. The two nuclei in the chalazal end of the four-nucleate sac become the "antipodals" of the mature sac. They do not divide, but become somewhat enlarged. They are nearly always in close contact with each other, and by the time of the origin of the embryo they may be partially fused. Still later they may become wholly fused (fig. 30), while in other cases they never unite, but remain separate until a very late period in the formation of the endosperm and embryo, when they finally disappear. Coincidentally with the downward growth of the sac, the greater portion of the cytoplasm and the two micropylar nuclei, together with most of the food substances, move into the tubelike extension as it is being formed. This mass usually lies a short distance from the apex of the sac as it works its way up through the tissue of the carpel (figs. 32, 33). The cytoplasm which is in contact with the wall of the micropylar end of the sac does not contain starch. It is very dense, finely granular, and stains more darkly than the cytoplasm of the lower portion of the sac, in which the dividing nuclei and food substances are imbedded. With iodine it stains a yellowish dark brown. It is evident that this is a specialized portion of the protoplasm of the sac which probably secretes an enzyme for digesting the tissue as it advances through the carpel. This is evident from the fact that a number of examples were found where the apex of the sac extended up between the cells of the tissue

adjacent to it in the form of pseudopodium-like projections. The cells in contact with these pseudopodia were partially digested (figs. 34, 43). The nuclei of the long arm of the sac are derived from the two micropylar nuclei of the four-nucleate stage, at which period in the development of the sac they are usually a short distance apart. Preceding their division, which begins about the time the sac commences to grow downward, they come together and lie in close contact until after they have divided. They are so intimately associated that in some examples they appear to be partially fused. In the stage preceding the upward growth of the tubelike sac, they are found partially divided in a mass near the tip of the sac, in which 6 nucleoli are distinguishable, some of which are entirely inclosed by a nuclear wall (figs. 35, 36, 38, 39). Examples were found where the sac had advanced well up into the carpel and in which the nuclei had just begun to divide (fig. 34). Either 5 or 6 nuclei are formed, and for some time after the divisions are complete they lie massed together (figs. 32, 37). About the time the tip of the sac has reached to or a little above the level of the apex of the axis, the nuclei of the sac separate, 2 occupying the position of polar nuclei, the other 3 or 4 forming the egg apparatus (figs. 41, 42, 43). The two nuclei corresponding to polar nuclei of a sac of the usual type are sister nuclei, having resulted from the division of one of the two nuclei at the micropylar end of the four-nucleate sac. From the sister nucleus of this same nucleus the nuclei of the egg apparatus are derived. There are 7 or 8 nuclei formed in each sac. If we try to homologize them with the nuclei of a sac of the usual type, we find 2 or 3 cells having the position of synergids accompanying the egg nucleus at the micropylar end of the sac, the 2 just below the egg have the position of polar nuclei, and the 2 in the chalazal region correspond to antipodals. While the long arm of the sac has been developing, the chalazal end has been enlarging and slowly advancing toward the apex of the placenta where it meets the chalazal arm of the sister sac (text fig. 20). At first they are separated by a thin wall, which finally disappears during the early stages of the formation of the embryo, so that the two sacs form one continuous tube (fig. 30).

The embryo sac of *Dendrophthora* as thus seen is quite different

from that known in other Loranthaceae. In *Viscum album*, *V. articulatum*, and *Arceuthobium Oxycedri* the sacs are similar to the common type of embryo sac. In *Loranthus sphaerocarpus* the sac is long and tubular, extending up in the narrow stylar canal. In the two species of *Dendrophthora* which are the subject of this study and *Arceuthobium Oxycedri* the embryo sacs originate in a quite similar manner in the elongated floral axes, which also bear a strong resemblance in general form. The sac in the latter species grows up to the apex of the axis where it is met by the pollen tube. As already shown, there is a strong similarity between the megasporangia of *Dendrophthora*, *Thesium*, and *Santalum*. Also the sacs of *Dendrophthora* and *Santalum* are much alike in their general shape and behavior. In the latter the sac grows downward, curves, and extends upward just outside of the "mamelon." The similarity between the genera of the Santalaceae mentioned above and *Dendrophthora* in the position of the megasporangia and development of gametophytes might be taken as indicative of a phylogenetic relationship.

Of the two sacs formed in each flattened "mamelon," the one toward the apex of the spike becomes functional. The preceding description applies to this sac. It is somewhat larger and usually develops a little more rapidly than its mate. As a rule, the long arm of the latter extends a short distance above the level of the apex of the "mamelon." The development and arrangement of its nuclei are the same as in the micropylar end of the functional sac. Only a few examples of 4 nuclei in the egg apparatus were found.

The divisions of the nuclei of the sac of *Dendrophthora gracile* are usually completed much earlier than those of the sac of *D. opuntiioides*. Examples were found in which the nuclei of the long arm of the sac were formed by the time the sac had begun its downward growth (figs. 44, 45). Aside from this difference in time of division the embryo sacs, these two species of *Dendrophthora* are essentially alike. No traces of mitotic divisions of the embryo sac nuclei subsequent to that of the megaspore mother cell were observed until after the maturation of the sac. The chromatic material of the nucleus during this period appears to

have become concentrated into a single nucleolus-like body, which stains uniformly and reacts with Flemming's triple stain, Haidenhain's iron alum hematoxylin, cyanin and erythrosin, thionin, and methyl green and acid fuchsin, as chromosomes do in ordinary mitotic cell division. The division of the nuclei apparently always begins by a fission of this nucleolar mass, which is followed by a constriction of the nucleus (figs. 39, 40). This mass divides into a number of parts equal to the number of nuclei to be formed. For example, in the division of the parent nucleus of the egg apparatus, the nucleolus divides into 3 or 4 parts, each of which later becomes inclosed within a nucleus (figs. 36, 38). The staining reaction and the mode of division of the nucleolus-like body of the nucleus as thus seen clearly indicates that it is composed of chromatin, and instead of dividing into the same number of chromosomes as occur at the time of the division of the megaspore mother cell, it divides only into 3 or 4 parts. Judging from all appearances, the nuclei during the above mentioned period divide amitotically. The manner of the formation of the gametophytic nuclei is apparently quite unique, as the author has found thus far no indication in the literature that such a phenomenon has hitherto been observed in the development of an embryo sac. The mode of division of these sac nuclei is probably stimulated in some way by the presence of abundant food material within the sac.

A number of examples were found where 1-3 small bodies, which stained like the chromatic material in the nuclei of the same embryo sacs in which they occurred or as described in connection with figs. 39, 40, were present in the terminal portion of the embryo sac in the dense finely granular protoplasm mentioned above (figs. 32, 33, 37). It was not possible to discover their origin or fate.

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EXPLANATION OF PLATES V AND VI

All figures, with the exception of figs. 8, 12*a*, 20, and 21, which are diagrams, are camera lucida drawings from microtome sections. Figs. 3, 6, 22, and 66 were made with a Bausch and Lomb dissecting microscope. In making the remainder of the drawings a Leitz compound microscope was used.

Abbreviations: *a*, floral apex; *an*, antipodals; *b*, bracts; *c*, swollen collar

of the inflorescence axis; *ca*, carpels; *ce*, cutinized epidermis; *cw*, wall of carpels; *d*, definitive nucleus; *dp*, degenerating polar nuclei; *e*, egg nucleus; *ea*, nuclei of egg apparatus; *em*, embryo; *en*, endosperm; *f*, young flower or floral apex; *fn*, fusion nucleus; *i*, rudimentary integuments; *m*, mamelon; *n*, nucellus; *p*, polar nuclei; *pc*, fleshy pericarp; *pl*, placenta; *pi*, pistil; *r*, remains of nuclei of egg apparatus; *s*, stem; *se*, sepal; *sn*, synergid; *st*, starch; *v*, vascular bundle; *vf*, very young flower; *x*, nuclei from which nuclei of egg apparatus and polar nuclei are derived; *y*, chromatin-like bodies.

FIGS. 1, 7, 8, 12a, 20, and 21 are text figures.

FIG. 2.—Part of longitudinal section of apex of young spike; $\times 100$.

FIG. 3.—Lateral view of young spike; $\times 1.5$.

FIG. 4.—Part of longitudinal section of apex of spike showing very young flower; $\times 26$.

FIG. 5.—Part of cross-section of young spike showing initiation of flower from periblem; $\times 100$.

FIG. 6.—Lateral view of large spike showing sequence of development of flowers; $\times 1.5$.

FIG. 9.—Outline of cross-section of spike showing sunken flower before sepals have begun to form; $\times 26$.

FIG. 10.—Outline of cross-section of spike showing sunken flowers with sepals; $\times 26$.

FIG. 11.—Longitudinal section of flower showing sepals and incipient carpels (*ca*); $\times 26$.

FIG. 12.—Longitudinal section of terminal portion of inflorescence axis showing young flowers; $\times 10$.

FIG. 13.—Outline of longitudinal section of flower at two-nucleate stage of embryo sac; $\times 26$.

FIG. 14.—Transverse section of "mamelon" at two-nucleate stage of embryo sac; $\times 100$.

FIG. 15.—Outline of longitudinal section perpendicular to broad surface of "mamelon" at two-nucleate stage of embryo sac; $\times 100$.

FIG. 16.—Section of part of "mamelon" showing megaspore mother cell with subjacent cells (*n*) which give rise to nucellar tissue; $\times 150$.

FIG. 17.—Longitudinal section of "mamelon" in plane of flattening, showing two-nucleate sac, nucellar tissue, and placenta; $\times 150$.

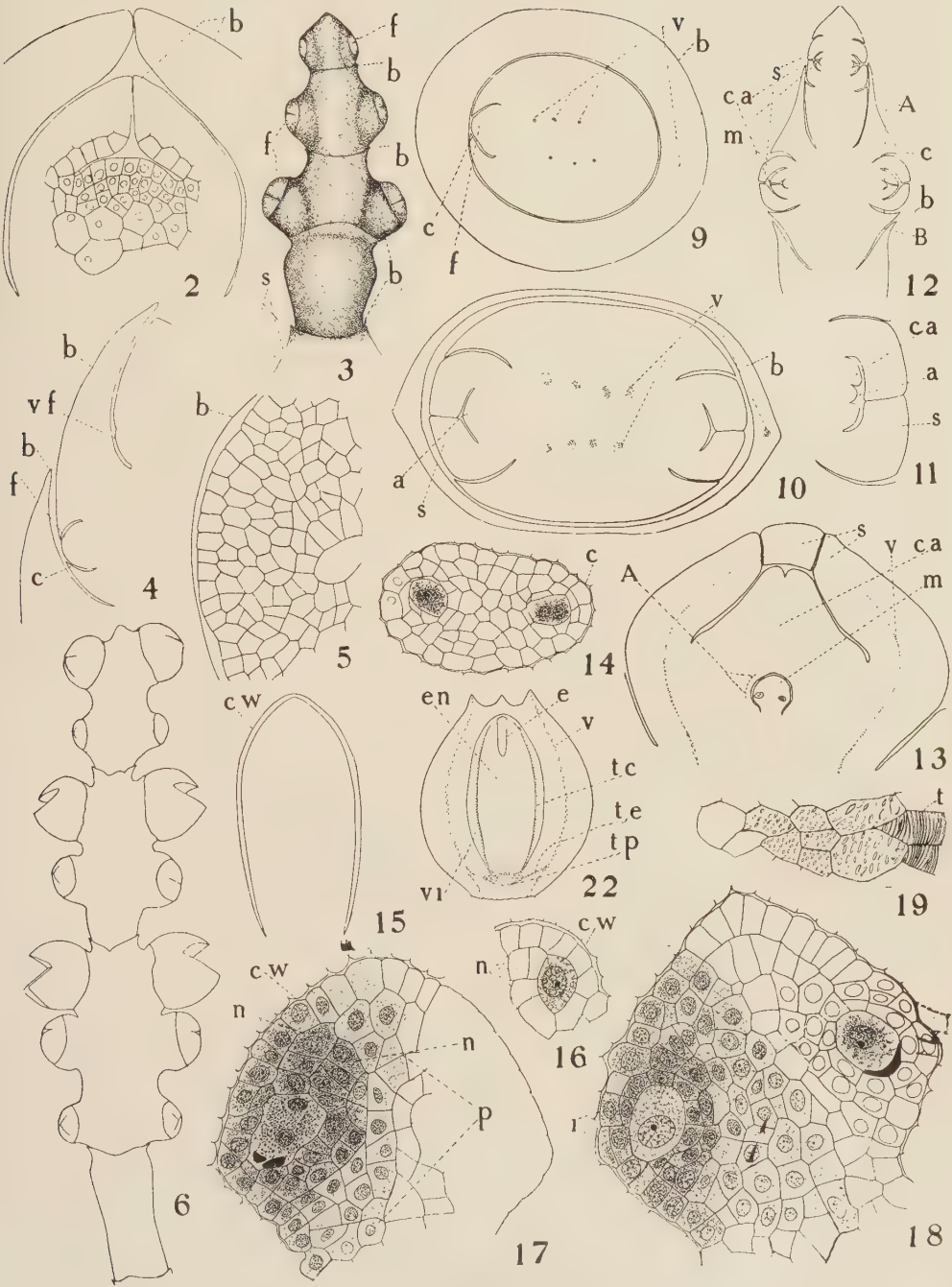
FIG. 18.—Longitudinal section of "mamelon" of *D. gracile* in plane of flattening, showing uninucleate embryo sac and rudimentary integuments (*i*); $\times 150$.

FIG. 19.—Portion of section of tracheid tissue at base of "mamelon"; $\times 250$.

FIG. 22.—Longitudinal section of ripe berry showing embryo, endosperm, pericarp, and vascular system: *tc*, *v*, *t*, *tp*, as in fig. 21; $\times 1.75$.

FIG. 23.—Section of "mamelon" showing archesporial cells; $\times 100$.

FIG. 24.—Part of section of "mamelon," showing megaspores; $\times 150$.



YORK on DENDROPHTHORA



YORK on DENDROPHTHORA

FIG. 25.—Part of section of “mamelon,” showing disintegration of basal megaspore; $\times 150$.

FIG. 26.—Longitudinal section of two-nucleate embryo sac; $\times 150$.

FIG. 27.—Longitudinal section of four-nucleate sac; $\times 150$.

FIGS. 28, 29.—Longitudinal sections of “mamelon,” showing one of the embryo sac cavities filled with starch; $\times 100$ and 26 respectively.

FIG. 30.—Longitudinal section of flower of *D. gracile*, showing fusion of short arms of embryo sacs, resulting in formation of a single tube; *tp* same as *tp* in fig. 20; $\times 150$.

FIG. 31.—Part of longitudinal section of that portion of the carpel taken at *A* in fig. 13; $\times 150$.

FIG. 32.—Longitudinal section of upper portion of embryo sac, showing polar nuclei, nuclei of egg apparatus, and three chromatin-like bodies (*y*) in terminal portion of sac; $\times 250$.

FIG. 33.—Longitudinal section of upper portion of embryo sac showing chromatin-like bodies (*y*) and group of nuclei from which those of egg apparatus and polar bodies are to be derived; $\times 250$.

FIG. 34.—Longitudinal section of young embryo sac, showing pseudopodium-like projections; $\times 150$.

FIG. 35.—Longitudinal section of young embryo sac which has begun to grow downward, showing dividing nuclei; $\times 250$.

FIG. 36.—Detail drawing of nuclei *ea* and *p* in fig. 35; $\times 500$.

FIG. 37.—Nuclei at micropylar end of sister sac to the sac shown in fig. 35; $\times 500$.

FIG. 38.—Dividing nuclei at micropylar end of sac from which the synergids, egg, and polar nuclei are to be derived; $\times 500$.

FIGS. 39, 40.—Dividing nuclei showing segmentation of the nucleoli; $\times 500$.

FIG. 41.—Longitudinal section of terminal portion of embryo sac; $\times 500$.

FIG. 42.—Cross-sections of mature embryo sac of *D. gracile*; $\times 150$.

FIG. 43.—Longitudinal section of mature embryo sac, showing three synergids, egg, and degenerating polar nuclei; $\times 150$.

FIGS. 44, 45.—Longitudinal section of embryo sacs of *D. gracile* that have just begun to go downward; $\times 250$.

THE ORIGIN AND DEVELOPMENT OF THE EMBRYO
SAC AND EMBRYO OF DENDROPHTHORA
OPUNTIOIDES AND D. GRACILE. II

HARLAN HARVEY YORK

(WITH PLATE VII)

Embryo-formation in *D. opuntioides*

Following the arrangement of the nuclei in the micropylar end of the sac, as described above, they enlarge, the one toward the so-called polar nuclei becoming the largest. This nucleus becomes the one functional cell from which the endosperm and embryo are derived. Later the "synergid" and the polar nuclei begin to degenerate and in a short time disappear. Preparatory to division, the functional nucleus becomes very much enlarged and divides by ordinary mitosis, in a plane transverse to the longitudinal axis of the sac (fig. 46). This mitotic division was the first observed following that of the megaspore mother cell. It was not possible to count the chromosomes, but the number was apparently equal to that seen at the division of the megaspore mother cell. The two cells resulting from the division of the functional nucleus divide transversely to the plane of the first division. After a series of divisions a large oval mass of tissue is formed which occupies the upper end of the sac. The cells of this body are large and appear to be exactly alike (fig. 47). After this endosperm-like body has reached about one-twentieth of its size in the mature seed, the cytoplasm of one of the central cells becomes more densely granular than in the others. This cell constitutes the one-celled stage of the embryo (fig. 48). It divides by a wall almost transverse to the longitudinal axis of the flower, forming a 2-celled embryo (fig. 49). A division of these two cells in a plane transverse to the wall which separates them results in the formation of the 4-celled embryo (fig. 50).

The embryo thus arises indirectly from a single cell without fertilization having occurred. The author examined carefully more than 500 plants of *D. opuntioides*, occurring within a radius

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of 5 or 6 miles around Cinchona, Jamaica, at altitudes varying from 2000 to 5500 ft. above sea-level, and could find no staminate flowers. Microtome sections of more than 200 ovules mature enough to show whether pollination had occurred gave no traces of pollen adhering to the stigmas or pollen tubes within the tissue of the style. Examination of 150-200 ovules of *D. gracile* likewise failed to show pollen tubes. If pollination does occur in these plants, the mode of origin of the embryo and endosperm is strikingly different from anything elsewhere known. In recent studies on *Phoradendron flavescens*, the author observed near Austin, Texas, a single plant more than a mile distant from others of its kind, fruiting abundantly. The berries of this plant contained normal seeds. Since *Phoradendron flavescens* is dioecious, the chances for wind pollination in this case were very slight. Hence it is apparent that this plant was producing fruit without having been pollinated. In the following year a number of branches bearing pistillate flowers were covered with cheese-cloth bags before the shedding of the pollen had begun, and allowed to remain until there was no longer any possibility of pollination. They produced fruit as abundantly as the uncovered branches. Thus it is evident that in *Phoradendron flavescens* pollination is not necessary for the production of seeds. In fact, pollination probably does not occur in plants under natural conditions, for in studying sections of a few hundred young fruits of this species, no pollen tubes or germinated pollen were seen. There are 18-22 chromosomes present in the cells of the embryo of *D. opuntiioides*, which is the number found at the division of the megaspore mother cells. There is thus apparently a constant number of chromosomes throughout all the different stages of development of this plant, which seems clearly to preclude all possibility of fertilization. Since no nuclear fusions were seen within the sac, it seems evident that no reduction division has occurred previous to the formation of the gametophyte. Estimates of chromosomes made in the cells of the embryo of *D. gracile* show that there are 18-20. The cells of the endosperm in each species contain the same number of chromosomes as is found in the embryo.

It was impossible to obtain a complete series of good sections of the different stages in the development of the endosperm and

embryo, owing to shrinkage of the material from lack of penetration of the fixing fluids. The growth of the embryo proceeds very slowly as compared with the endosperm, and it is but a small spherical mass of cells when the latter has come to occupy the whole central region of the carpellary tissue (fig. 51). At first the embryo is intimately connected with the endosperm, from which it gradually separates as development continues. The micropylar end or the radicle is first freed, while the cotyledons are last to be separated. The embryo is strictly dicotyledonous, and when mature is almost entirely imbedded within the endosperm (fig. 52). The endosperm enlarges at the expense of the adjacent tissues. Soon after the origin of the embryo, it begins to elongate in the direction of the longitudinal axis of the ovary and at the same time becomes flattened in the plane of flattening of the spike. Growth at first is almost entirely upward and the apex of the endosperm reaches to the base of the style. Later, by gradually digesting the remains of the placenta, it advances downward into the plate of tracheids at the base of the ovary. The endosperm thus replaces the entire axial region of the flower and lies in direct contact with the vascular traces of the carpels (figs. 20, 21, *tc*) which later form a part of the covering of the seed (figs. 22, 66).

The endosperm is composed of large parenchyma cells, the walls of which are relatively thin and of a most uniform thickness. The cells of the external layer are largest, attaining their greatest size at the base of the endosperm (figs. 53, 54). They are not specially modified for protection as in *Phoradendron flavescens*, and do not form a true epidermis. They fit loosely together and seem to have an absorptive function. This is especially true in the basal portion of the endosperm, in which the outermost layer of cells contains large nuclei and very fine granular cytoplasm which stains brownish yellow in iodine (fig. 53). Since these cells are in direct contact with tracheid cells, the greater bulk of food passing into the endosperm must enter through them. Hence they are considered as being primarily absorptive in function. The fact that the cells of the outer layer fit loosely together also indicates that they are capable of readily absorbing water when germination begins. Sections of seeds which had just begun to germinate showed that

these cells had become turgid and crowded closely together into a very compact layer (fig. 55).

In the ripe seed the outer layer of cells contains large quantities of a fatty substance which stains black in osmic acid and dark blue in cyanin (figs. 53, 54). When germination begins, the fat, as indicated in figs. 53 and 54, almost entirely disappears from them and they become practically colorless. Large brown cystoliths, the chemical nature of which was not determined, were found in some of the cells. The remaining cells contain starch and other food substances which are more or less limited to certain portions. Fatty substances as seen in the outer cells also occur in the inner region of the endosperm and are in greatest abundance in the chalazal part (fig. 53). There is a single layer of cells, densely filled with chloroplasts and starch, subjacent to the outer cells (fig. 55, *cl*). The cells adjacent to the cotyledons (fig. 55, *i*) contain practically no chlorophyll and very little starch, but substances which stain like proteins. Immediately above this layer the cells are abundantly filled with starch (fig. 55, *z*). The remaining portion contains less starch and chlorophyll, but in addition has large cystoliths of calcium oxalate (fig. 55, *cs*). The cells of the embryo are also well supplied with chlorophyll. When germination begins, the chlorophyll present in the seed becomes greatly intensified. It is important to note in this connection that chlorophyll is most abundant in the cells just below the outer layer of cells which become almost colorless when germination occurs. The chlorophyll in the endosperm is manifestly for photosynthetic work, as a result of which food is furnished to the young plant until it becomes fixed on its host. It becomes more evident that the chlorophyll performs the work of photosynthesis from the fact that the seeds will not germinate in the dark nor will germinating seeds continue to develop if kept from the light. A number of attempts to germinate seeds of *D. opuntiioides* in a dark chamber in the laboratory were made, but all failed. Also small dark chambers were constructed out of black paraffined paper over seeds that had been dropped on the branches by birds. At the end of four weeks these seeds had not germinated, while uncovered seeds near those which were covered had germinated. Seeds of *Den-*

dropemon parvifolius were placed in the dark chamber with those of *Dendrophthora opuntioides* and germinated as readily as those under normal conditions. Chlorophyll is not present in the endosperm of *Dendropemon parvifolius*, but is abundant in the embryo. From this fact it is seen that the endosperm in *Dendrophthora* and also in *Phoradendron*, which is quite similar, is also a carbon dioxide assimilating organ. It will be of interest to mention here that the radicles of the seedlings of *Dendropemon parvifolius* which germinated in darkness did not turn down toward the substratum, but grew out at various angles. Some, for example, extended upward at an angle of 90° , while others were parallel to the substratum. Thus the bending of the radicles toward the substratum is not due to gravity or to attraction by the substratum, but their behavior seems to be a negative response to the stimulus of light.

DUTROCHET (6) found that the "hypocotyls" of *Viscum album* are negatively heliotropic. His observations were later confirmed by WIESNER (39). The endosperm in its development, as seen in this study, may thus be regarded as being parasitic, drawing its nourishment through its outer layer of haustorial cells from the adjacent tissues. It also becomes a storage tissue, containing an abundance of food material for the embryo and a region in which photosynthesis occurs.

The viscin

Owing to the lack of fresh material at Baltimore, a detailed study of the viscin has not been possible. Its structure is essentially the same as described and figured by JOHNSON in *Arceuthobium Oxycedri*, and by PEIRCE in *A. occidentale*. Parallel with the maturation of the embryo sac, the cells in the outer half of the carpels become colorless and begin to enlarge (figs. 20, 56). Later they elongate obliquely toward the base of the style when the formation of the embryo begins. In the ripe berry they are greatly elongated and their walls consist of two layers. The outer layer is composed of gelatinous material, while the inner is usually made up chiefly of spiral cellulose thickenings (fig. 57). As a rule the cell cavity is very narrow, and in some cases has been almost obliterated, the cell being chiefly composed of gelatinous material (figs. 58, 59). In some cells the outer wall is spirally thicken-

The inner ends of the cells are attached to a sort of membrane which is two or three cell layers in thickness. PEIRCE has described a similar structure, a "sclerotic membrane," in *Arceuthobium occidentale* as being composed of three or four layers of tannin cells. Apparently there was no tannin in the cells of this body in *Dendrophthora opuntoides*. The viscin in *Phoradendron flavescens* presents essentially the same structure as that here just described, though this was not recognized by the author in his earlier studies on this plant. The layer of viscin surrounding the seed in *Dendrophthora* is much less in proportion to the size of the berry than in *Phoradendron flavescens*. In the latter the viscin evidently prevents extreme drying out and is useful in absorbing water before germination begins. It is not necessary for the germination of the seeds, but if it is stripped from them they will dry up in a few weeks' time when exposed to a dry atmosphere. Seeds from which the pulp has not been removed retain their vitality for an indefinite period of time. In January 1909, the author collected in Austin, Texas, berries of *Phoradendron flavescens* which were dried at room temperature. During June 1910, 12 of the dried berries were soaked in water for 24 hours, after which a part of the pulp was removed and the seeds were placed in a moist chamber. At the end of two weeks 4 of the seeds had begun to germinate. Owing to the warm moist environment of *Dendrophthora opuntoides*, the pulp is probably not an important factor as a means of protection and aid in germination of its seeds, since they germinate almost immediately after being deposited.

The mature berries are bright red in color, oval in shape, 7-9 mm. long, and 6-8 mm. in diameter. Owing to the peculiar order of origin of the flowers, various gradations in the development of fruits, from flowers to ripe berries, often occur in the same spike. The seed is enveloped by a thin layer of viscin, which is surrounded by a thick fleshy pericarp. The latter is covered by a heavily cutinized single-layered epidermis (figs. 22, 66).

Embryo-formation in *D. gracile*

The micropylar nuclei of the sac enlarge as much as they do in *D. opuntoides*. Meanwhile the two polar nuclei, which are

located some distance below them, are fusing and migrating upward in the sac (fig. 60). The nucleus resulting from their union lies just below the egg and becomes the nucleus of the primordial cell of the proembryo (figs. 61, 62). Before division it enlarges greatly and is apparently cut off from the sac below by a transverse wall. At the same time two of the micropylar nuclei, the synergids, begin to degenerate. The other nucleus, which is homologous with the one in the sac of *D. opuntioides* from which the proembryo originates, continues to enlarge for a short time as if preparing to divide, and then also begins to degenerate before division of the fusion nucleus occurs. The primordial cell of the proembryo divides by ordinary mitosis (figs. 62, 63). The two resulting cells (fig. 64) by further divisions form a proembryo, which is similar to that of *D. opuntioides* (fig. 65). The development which now follows is also the same as has been given above, hence further descriptions are unnecessary. The origin of the embryo as seen in *D. gracile* suggests the example described by FARMER and DIGBY (8) in *Lastrea pseudo-mas* var. *polydactyla* Wills, in which the embryo arises from a nucleus resulting from the union of two body cells of the prothallus which has come from a normally developed spore and has the haploid number of chromosomes. The difference in the mode of origin of the embryos of *D. gracile* and *D. opuntioides* also recalls that shown by the above mentioned authors between *Lastrea pseudo-mas* var. *polydactyla* Wills and the closely related *Lastrea pseudo-mas* var. *polydactyla* Dadds. In the latter the embryo arises from a projection or budding out of the prothallus which has arisen aposporously. In this same variety these authors also found nuclear fusions occurring in the prothalli which had come from normal spores, quite similar to those in the variety first mentioned. It is probable that a phenomenon similar to that just stated for Dadds' fern occurs in *D. opuntioides*, though no evidence for such a supposition was seen. Owing to the lack of material, it was not possible to make a detailed study of the division of the megaspore mother cell. At the division of the microspore mother cell in *D. gracile* 9 chromosomes were found passing to each pole of the spindle; 18-20 chromosomes were estimated in the cells of the developing embryo. Apparently the same number is also present

in the nuclei of the endosperm. From these facts it is clearly evident that fusion of the polar nuclei in *D. gracile* indicates that the nuclear behavior in the origin of the female gametophyte is the same as in an ordinary sexual plant.

Discussion

The embryos of *D. opuntiioides* and *D. gracile* arise asexually. In the former, reduction division does not occur preceding the formation of the gametophyte, the nuclei of which thus contain the diploid number of chromosomes. The gametophyte is aposporous in origin. Examples of apospory in seed plants have been described by JUEL (16) in *Antennaria alpina*, by MURBECK (22) and STRASBURGER (32) in certain species of *Alchemilla*, by TREUB (34) in *Balanophora elongata*, by OVERTON (27) in *Thalictrum purpurascens*, by LOTSY (19) in *Balanophora globosa*, by OSTENFELD (25, 26), ROSENBERG (29, 30), and MURBECK in species of *Hieracium*, by WINKLER (40, 41) in *Wikstroemia indica*, and by RAUNKIAER (28), MURBECK (23), and JUEL (17) in a number of forms of *Taraxacum*. Apogamy is associated with the aposporous origin of the gametophyte in these forms. In each of these plants, with the exception of *Balanophora*, the embryo arises from what is to external appearances an egg. From a cytological point of view the studies of STRASBURGER and WINKLER are the most important. The former regarded the cell giving rise to the embryo as a sporophytic cell, since it possesses the diploid number of chromosomes. According to this view the embryo may be said to develop by vegetative budding. STRASBURGER regards the process as a case of apogamy and not parthenogenesis as some authors claim. He defines an egg as a cell having the haploid number of chromosomes and capable of being fertilized.

WINKLER disagrees with STRASBURGER in regard to the use of the terms apogamy and parthenogenesis and the theoretical significance of the chromosomes. According to him, apogamy is the origin of a sporophyte from some cell or group of cells of the gametophyte other than the egg. He uses the term parthenogenesis to indicate the formation of an embryo, developed without fertilization, from a cell having the position of an egg, whether the nucleus

of this cell is haploid or diploid. WINKLER also maintains that other factors besides the number of chromosomes determine the character of the gametophyte as well as the sporophyte. YAMANOUCHI's (42) studies on apogamy in *Nephrodium* support this hypothesis. If we accept STRASBURGER'S narrow definition of the egg, it seems logical to restrict the term gametophyte to structures having the haploid number of chromosomes, that is, to bodies developed after a normal sporogenesis. Regarding *Dendrophthora opuntoides* from this point of view, a true megaspore formation does not occur, the gametophytic generation itself is omitted, and the nuclei of the embryo sac are thus really vegetative or sporophytic in character. If the egglike cell in *Dendrophthora*, as well as in other similar cases, is a vegetative or sporophytic cell, it is strikingly different in behavior from all other sporophytic cells which we know.

I find no record among seed plants of the origin of an embryo from a cell of an endosperm-like mass, which like that of *Dendrophthora* has been derived from a single egglike cell. If this cell is really a vegetative cell of the sporophyte, we should expect it to develop directly into a sporophyte, as nucellar cells do in *Coleobogyne* and *Funkia*. In *Antennaria alpina* (JUEL 16) or *Thalictrum Fendleri* (DAY 4), for example, the egg cell which develops without fertilization should, if it is really a sporophytic cell, give rise to a sporophyte having the same sex as its parent. Furthermore, the derivation of the endosperm from a single polar nucleus is a peculiar phenomenon if this nucleus be regarded as sporophytic in character.

In the ferns, aposporous prothalli whose nuclei contain the sporophytic number of chromosomes are distinctly different in structure and in possessing sex organs from the parent sporophytes. Hence chromosome number cannot be the sole morphogenic factor in the nucleus. If the nucleus in any way determines structure, it is evident that the nuclei of an aposporous embryo sac must differ in some manner from those of the parent sporophyte, in spite of the identity in chromosome number. Morphologically the nuclei of such an embryo sac may be said to be equivalent in the same sense that the nuclei of a typical embryo sac are. The nucleus from which the embryo sac of *Dendrophthora opuntoides* develops,

however, is not a mere vegetative one, but is potentially distinct from all other nuclei in the sac. The functional nucleus in the sac of *D. opuntiioides* occupies the position of an egg in the usual type of embryo sac. It is different from a diploid egg in that it does not give rise directly to an embryo. It may thus be regarded as a pseudo-egg and the tissue developing from it may be considered a proembryo or pseudo-endosperm. Owing to the very wide range in meaning of the term endosperm, it is perhaps allowable to designate the proembryo as endosperm, although it is not endosperm in the strict sense. TREUB in his studies on *Balanophora elongata* found that one of the polar nuclei gives rise to a mass of tissue which is quite similar to the proembryo described above. He called this body endosperm. The embryo arises from one of the central cells of this body as in *Dendrophthora*. He asserts that the embryo is apogamous in origin. LOTSY arrived at the same conclusions from his studies on *Balanophora globosa*. WINKLER, agreeing with TREUB, designates this mode of origin of the embryo as "somatic apogamy," since the nuclei of the endosperm possess the diploid number of chromosomes. WINKLER applies this same term to the mode of origin of the embryo, for example in that of the *Lastrea pseudo-mas* var. *polydactyla* Dadds, in which the embryo arises by a budding out of the prothallus. Since in *D. opuntiioides*, as well as in *Balanophora*, the embryo does not arise directly from the gametophyte, it may be spoken of as pseudo-apogamous in origin.

The fact that there is a fusion of two nuclei in the sac of *D. gracile* preceding the formation of the proembryo is presumptive evidence that warrants the assumption that reduction has taken place in the formation of the megaspores. The nuclei of the sac have thus the haploid number of chromosomes. The nucleus occupying the position of an egg may or may not be capable of being fertilized. The two nuclei which occupy the position of polar nuclei are, as in *D. opuntiioides*, derived from the same nucleus. Such a fusion of sister nuclei is a phenomenon of unusual occurrence among plants. It is not unknown among animals. BRAUER (3) has shown that in the majority of parthenogenetic eggs of *Artemia salina* only one polar body is formed; a second one is occasionally

produced. This one, however, never passes out of the egg, but after migrating a short distance its nucleus returns to the egg nucleus and fuses with it, thus restoring the diploid number of chromosomes. BLACKMAN (2) believes that the nuclear fusion occurring in the aecidium of *Phragmidium* is a "reduced form of fertilization." The fusion of two gametophytic nuclei in *D. gracile* is indeed quite comparable to the union of the nucleus of one prothallial cell with that of another in *Lastrea pseudo-mas* var. *polydactyla* Wills, which FARMER and DIGBY designate as pseudo-apogamy. They state:

We would suggest the term pseudo-apogamy to cover instances in which the sexual fusion of gametes is replaced by a fusion of ordinary gametophytic nuclei which morphologically are not sexually differentiated. This would include the process as it occurs in the Uredineae and probably the Ascomycetes, and also the growing number of instances of apogamy in prothallia induced by cultural conditions, as well as such cases of obligate apogamy as that of the *polydactyla* varieties of the male fern.

According to them, conjugation of adjoining cells of the same filament in *Spirogyra* might be looked upon as examples of an analogous phenomenon. Whether the nuclei which fuse in *D. gracile*, in *Lastrea pseudo-mas* var. *polydactyla* Wills, and in *Spirogyra* really are undifferentiated morphologically is not definitely known. The gametes of *Spirogyra* or *Mucor*, for example, are believed to be morphologically alike. Aside from the fact that they are sometimes produced on different individuals, does not their conjugation constitute a process essentially like the examples just mentioned? Physiologically the gametes of *Spirogyra* and *Mucor* are believed to differ distinctly from each other. So in *D. gracile*, the author regards the two gametophytic nuclei which conjugate as potentially different from each other and from all other nuclei in the gametophyte. Externally they are alike. Therefore, may not their fusion constitute a process essentially like that of the conjugation of the gametes in *Spirogyra*? The union of the gametophytic nuclei in *D. gracile* does not result in the mixing of parental characters from separate individuals and does not constitute a true sexual process in this respect. Their fusion results in doubling the number of chromosomes and furnishes a stimulus to further development.

In these respects this process may be said to be like the usual type of fertilization. This conjugation of gametophytic nuclei in *D. gracile* may also in a sense be regarded as a sexual process, in that there is a gametophytic generation followed by a sporophytic in which the sexes are in different individuals.

DEBARY (5) was the first to use the term apogamy, restricting it to cases in which fertilization had disappeared;¹ hence this interpretation does not apply in *D. gracile*. The union of the polar nuclei in this plant should be regarded as a substitution process or pseudo-fertilization, which in some respects is like a true fertilization. The body arising from the fusion nucleus represents a proembryo or pseudo-endosperm, and the embryo is regarded as pseudo-apogamous in origin, as in *D. opuntioides*. The term pseudo-apogamy is not used here in the sense in which it was used by FARMER and DIGBY. In *Lastrea pseudo-mas* var. *polydactyla* Wills, there is a fusion of nuclei which the author believes is a substituted form of fertilization quite like that in *D. gracile*.

The presence of such a difference in the mode of origin of the proembryo in two closely related species as shown here is indeed a very striking phenomenon; perhaps, however, no more so than the two different modes of origin of the sporophyte in the varieties of *Lastrea pseudo-mas* already mentioned. This phenomenon may be due to the difference in the number of chromosomes present in the gametophytic cells. The fact that in one example development occurs without any sort of fertilization, while in the other it does not take place until the number of chromosomes is doubled, supports the theory that a nucleus must have the sporophytic number of chromosomes for starting the sporophytic generation. YAMANOUCHI's account of the origin of a sporophyte whose cells contain the haploid number of chromosomes from a haploid gametophyte in *Nephrodium* is evidence against this hypothesis. One of the most important problems for solution is the determination of the factors which have led to this difference. Since we know comparatively little of the physiological relation of the Lorantheae to their hosts, it is almost useless to attempt an explanation of this phenome-

¹ "Dass einer Species (oder Varietät) die sexuelle Zeugung verloren geht und durch einen andern Reproduktionsprozess ersetzt wird."

non. *D. opuntioides* and *D. gracile* usually occur on different species of plants, hence the food substances which they absorb are probably different in some respects. This difference in the character of food material may have given rise to the difference in the development of these two parasites. In view of the facts just mentioned, such an interpretation seems quite allowable, since the type of reproductive organs formed in *Saprolegnia* has been shown to depend upon the character of the medium in which it is grown.

Summary and conclusions

The general plane of branching of *Dendrophthora opuntioides* and *D. gracile* is isolateral.

The flower originates a short distance above the axil of the subtending bract.

The floral axis elongates, filling the entire ovarian cavity, and does not become attached to the carpels.

The ovules are greatly reduced, being practically naked nucelli, which are borne on a central placenta. There are two nucelli oppositely placed in the plane of flattening of the "mamelon." In a number of examples vestiges of integuments were found.

A single archesporial cell, hypodermal in origin, arises in each nucellus. The archesporial cell becomes the megaspore mother cell, which gives rise to two megaspores. The one toward the apex of the "mamelon" gives rise to an embryo sac.

Following the formation of the 4-nucleate stage of the embryo sac, the micropylar end of the sac grows almost straight downward until it extends below the level of the insertion of the placenta. It then curves outward into the tissue of the carpel, bends, and grows upward beneath the lining layer of cells of the ovarian cavity until the end of the sac lies almost above the apex of the "mamelon." The embryo sac is hook-shaped; the short arm of which lies within the "mamelon," the long arm in the tissue of the carpel. The short arms of the embryo sac fuse, forming one continuous tube.

There are 7 or 8 nuclei in each sac, 2 antipodals in the chalazal end of the sac, 3 or 4 in the egg apparatus, and 2 polar nuclei.

The 2 antipodal nuclei are sister nuclei and were originally the

2 nuclei present in the chalazal end of the 4-nucleate sac. The 2 polar nuclei are sister nuclei and are derived from one of the 2 nuclei present in the micropylar end of the 4-nucleate sac. The nuclei of the egg apparatus arise from the sister nucleus of the one from which the polar nuclei are derived.

The nuclei of the 2, 4, 7, or 8-nucleate stages of the embryo sac are apparently formed amitotically.

Pollination does not occur.

An embryo is formed in only one of the two sacs in each flower. In *D. opuntoides* the egg nucleus gives rise to a mass of tissue, the proembryo. From a central cell of the proembryo the embryo develops. The endosperm is derived from the remaining cell of the proembryo.

The embryo is regarded as pseudo-apogamous in origin.

In *D. gracile* the "polar nuclei" fuse and give rise to a proembryo quite similar to that in *D. opuntoides*. The embryo and endosperm arise in the same manner as in the latter.

The embryo is strictly dicotyledonous and is almost entirely imbedded in the endosperm.

Apparently there is no chromosome reduction preceding the formation of the megaspores in *D. opuntoides*, while in *D. gracile* this process does seem to occur. In the latter, the diploid number of chromosomes is restored at the fusion of the "polar nuclei."

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EXPLANATION OF PLATE VII

FIG. 46.—Longitudinal section of two-celled proembryo; $\times 150$.

FIG. 47.—Longitudinal section of proembryo; $\times 150$.

FIG. 48.—Longitudinal section of proembryo of *D. gracile*, showing one-celled embryo; $\times 150$.

FIG. 49.—Longitudinal section of proembryo with two-celled embryo; $\times 150$.



FIG. 50.—Longitudinal section of proembryo with four-celled embryo; $\times 150$.

FIG. 51.—Outline of longitudinal section of endosperm with inclosed embryo of a young seed, taken from young berry about the size of berry *A* in fig. 66; $\times 26$.

FIG. 52.—Outline of longitudinal section of endosperm with embryo from mature seed, showing distribution of oil globules; $\times 17.5$.

FIG. 53.—Portion of longitudinal section of endosperm from mature seed taken at *B* in fig. 52; $\times 150$.

FIG. 54.—Portion of longitudinal section of endosperm from mature seed taken at *A* in fig. 52; $\times 150$.

FIG. 55.—Portion of longitudinal section of germinating seed taken in region *C* in fig. 52; *cl*, cells abundantly supplied with chloroplasts; *cs*, cells containing cystoliths of calcium oxalate, starch, and small quantities of chloroplast; *z*, cells filled with starch; *e*, cells filled with protein-like substances; *ct*, cells of cotyledons of embryo; $\times 150$.

FIG. 56.—Part of longitudinal section of carpel taken at *O* in fig. 20, showing cells (*vi*) which give rise to viscin; $\times 150$.

FIGS. 57–59.—Portions of cells of viscin from ripe seed; $\times 150$.

FIG. 60.—Longitudinal section of micropylar end of embryo sac of *D. gracile*, showing fusion of polar nuclei; $\times 150$.

FIG. 61.—Longitudinal section of micropylar end of embryo sac of *D. gracile*, showing nucleus (*d*) formed by fusion of polar nuclei; disintegration of egg and synergid just beginning; $\times 150$.

FIG. 62.—Same as fig. 61, with fusion nucleus (*d*) of *D. gracile* preparing to divide; synergids and egg disintegrating; $\times 150$.

FIG. 63.—Division of fusion nucleus of *D. gracile*: probably displaced downward, from about level *A*, by fixation; the synergids and egg are disintegrating; $\times 250$.

FIG. 64.—Two-celled embryo of *D. gracile*: synergids have disappeared; egg disintegrating; $\times 150$.

FIG. 65.—Portion of longitudinal section of proembryo of *D. gracile*, which was composed of about 16 cells; $\times 150$.

FIG. 66.—Lateral view of spike with berries in various stages of development; also showing position of endosperm and embryo in relation to the system of arrangement of different members of plant; *B*, flower in which formation of proembryo has just begun; $\times 2.8$.

VITA

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